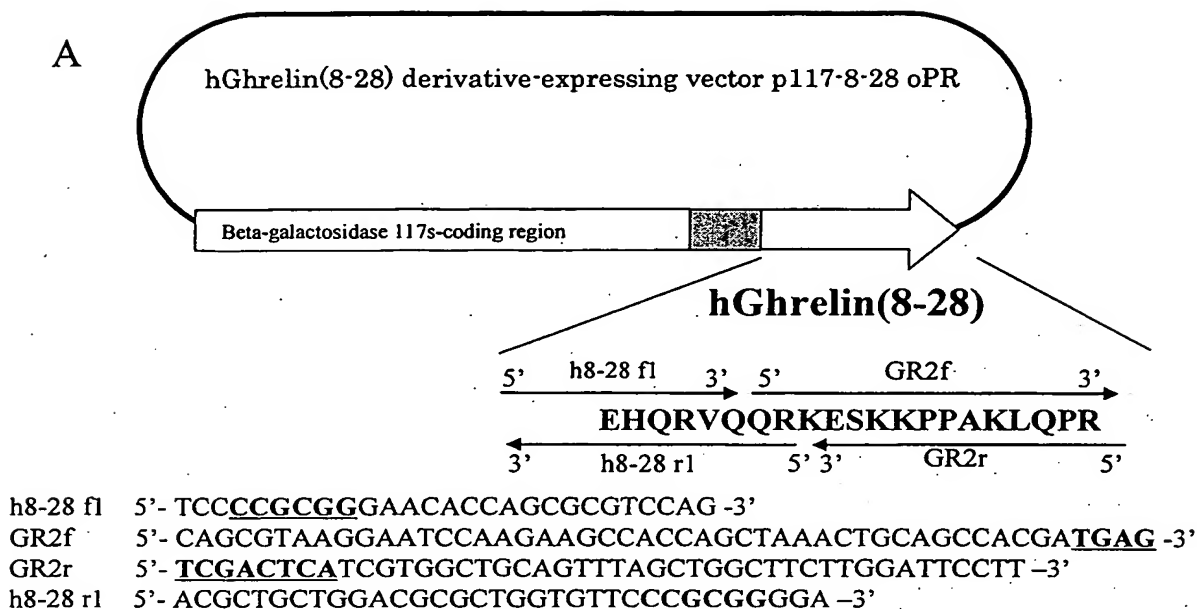



Fig. 1



Synthetic oligo-DNAs used in annealing method

- h8-28 f1, GR2f, h8-28 r1 and GR2r are synthetic oligo-DNA nucleotide sequence.
- Bold underlined bases in GR2f and GR2r sequences are termination codon and cohesive end of SalI cleavage site.
- Bold underlined bases in h8-28 f1 and h8-28 r1 sequences correspond to SalI cleavage site.

 corresponds to a linker sequence EPHHHHPGGRQMHGYDADVRLYRRHHGSGSPSRHRR.

B

Beta-Galactosidase 117s	Linker sequence	hGhrelin(8-28)
-------------------------	-----------------	----------------

Linker sequence EPHHHHPGGRQMHGYDADVRLYRRHHGSGSPSRHRR

hGhrelin(8-28) EHQRVQQRKESKKPPAKLQPR



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Fig. 2

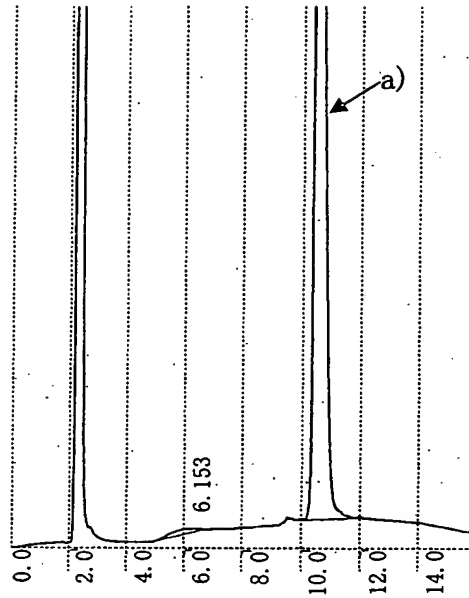


Fig. 3

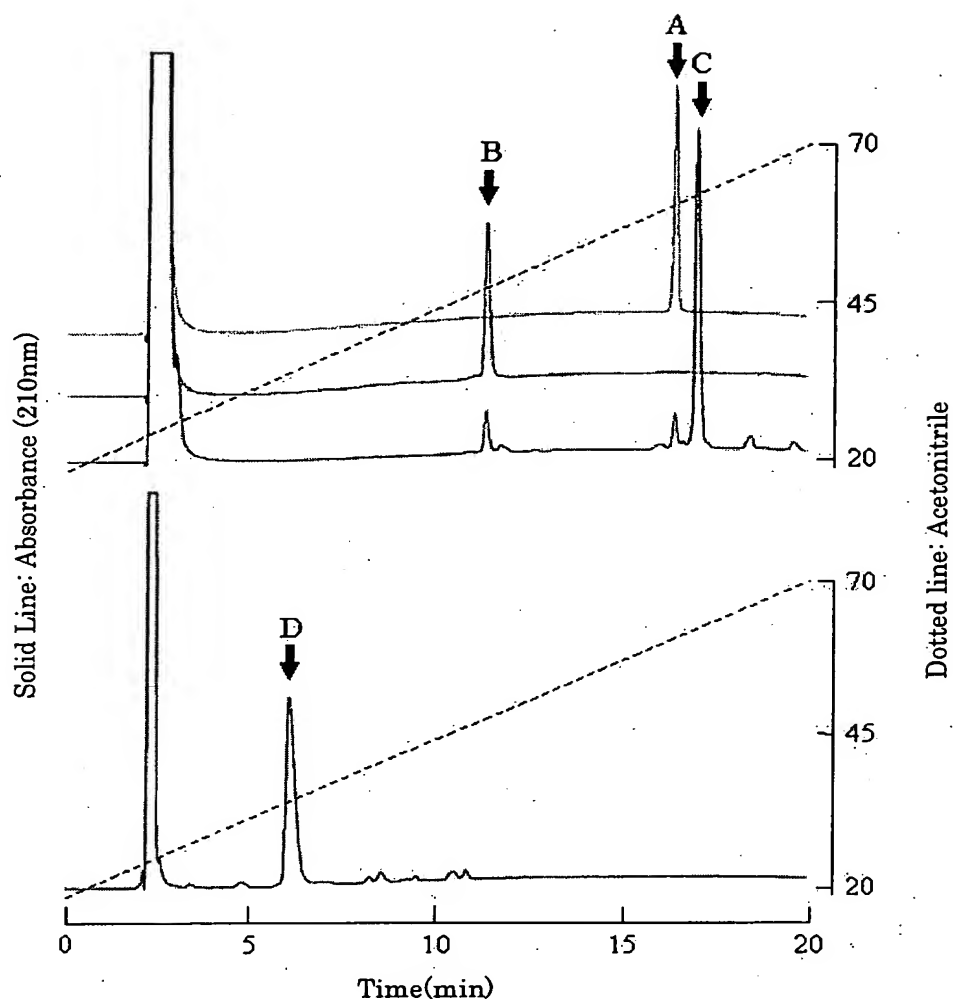




Fig. 4

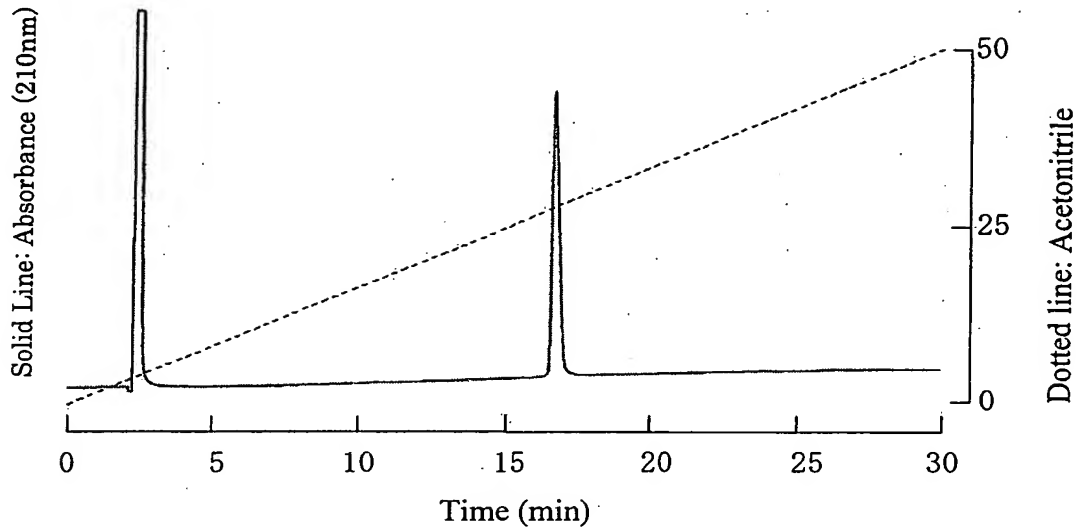
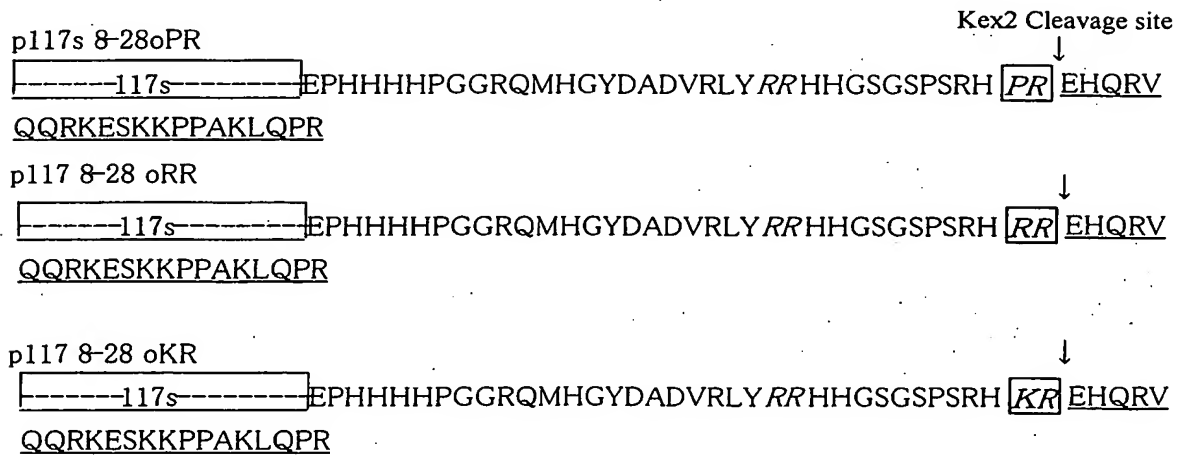


Fig. 5



-----117s----- is beta-galactosidase 117 amino acids

Underlined part is hGhrelin (8-28)

 represents a recognition site of Kex2, and ↓ represents a cleavage site of Kex2.



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Fig. 6

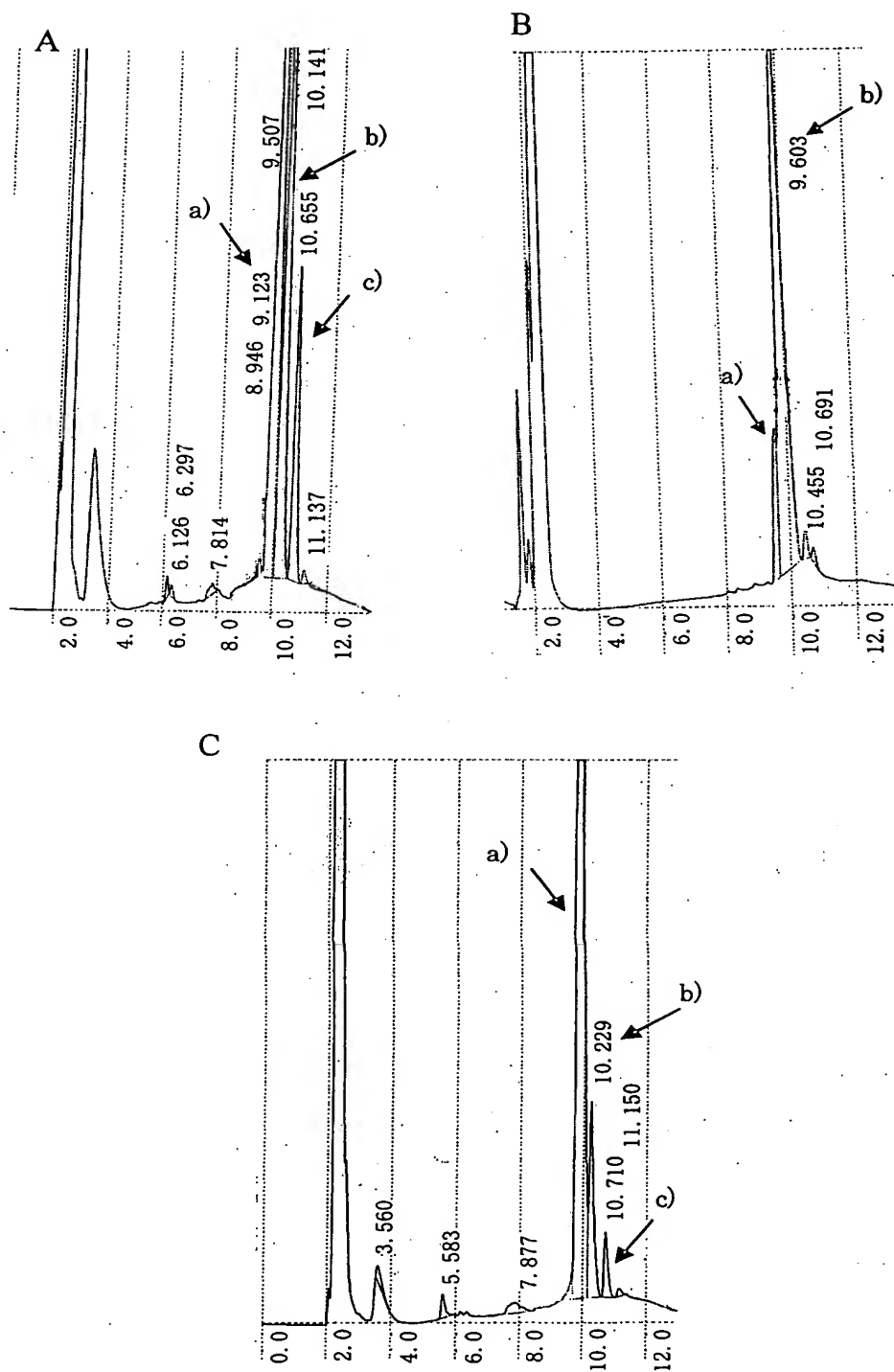
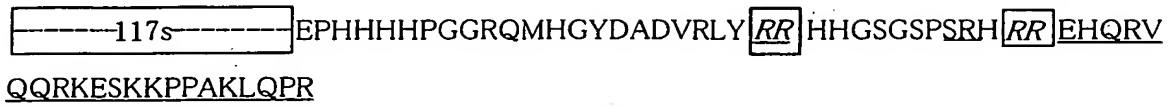


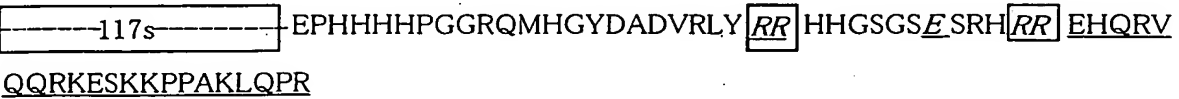
Fig. 7

117s 8-28 oRR

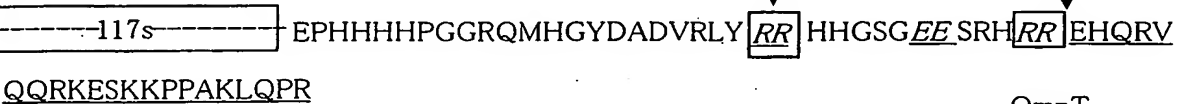
Fusion protein expressed in p117 8-28 oPR



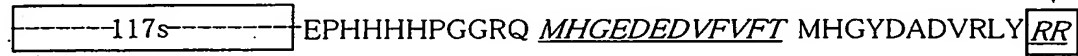
117s PE



117s SPEE



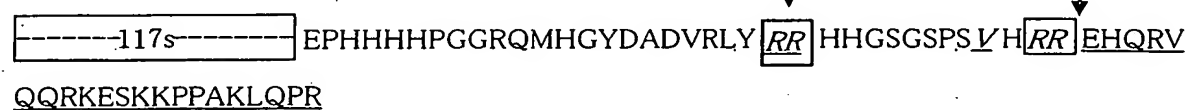
117s SPEE+12aa



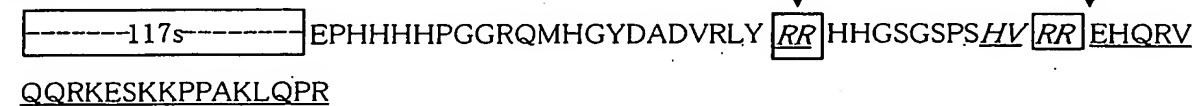
Kex2



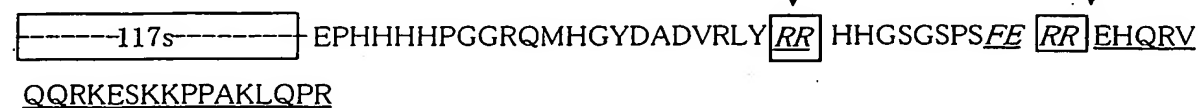
117s VH

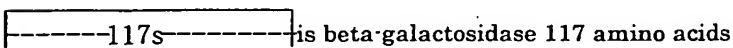


117s HV





117s FE





Underlined part represents hGhrelin(8-28).

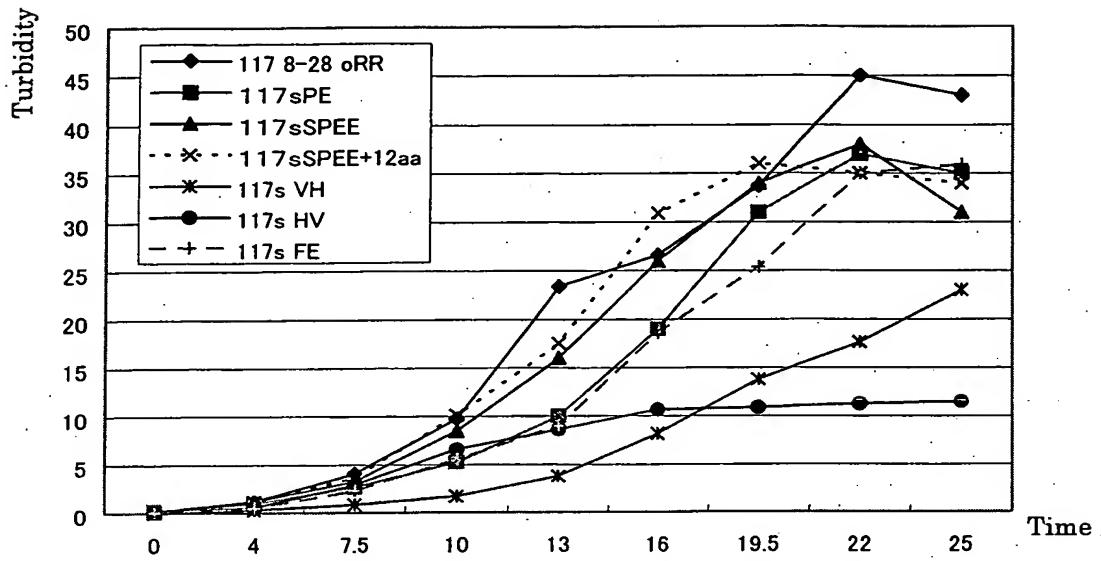
 and  represent cleavage recognition sites of OmpT and Kex2.

A slant underlined part represents a residue in which a mutation is introduced.



Fig. 8

A



B

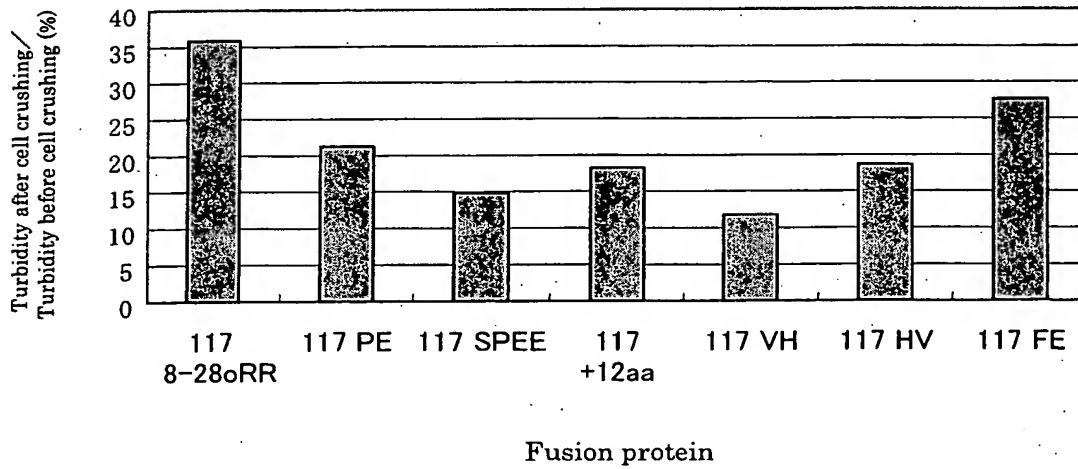


Fig. 9

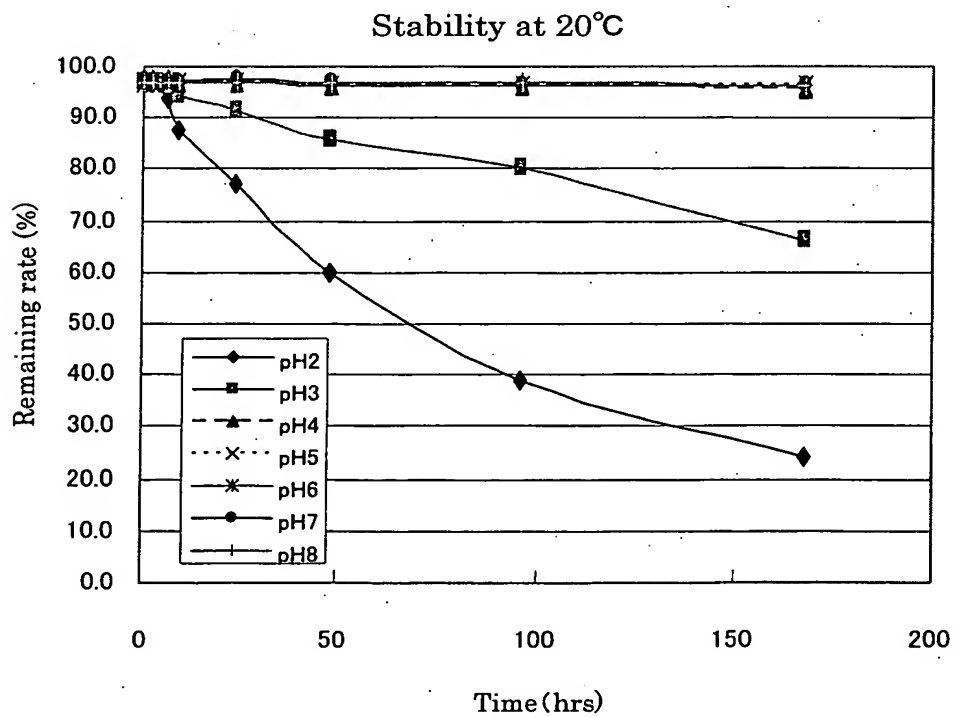
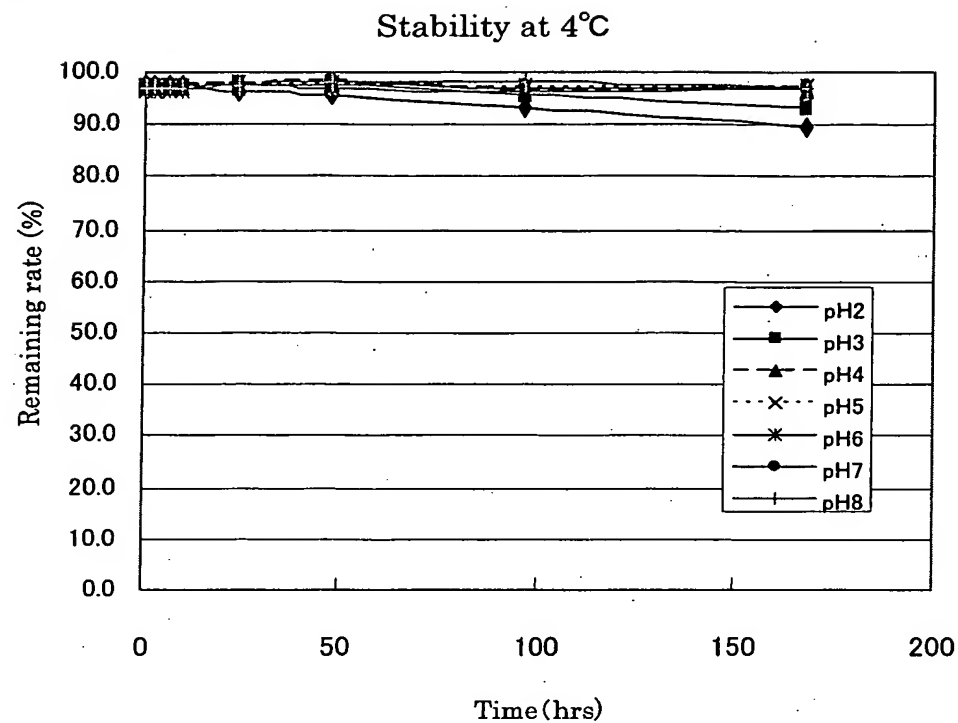




Fig. 10

